A cloned R gene from Phaseolus vulgaris confers a systemic necrosis response to Cucumber mosaic virus in Nicotiana benthamiana

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Bean dwarf mosaic virus (BDMV) is a single-stranded DNA virus (Genus Begomovirus, Family Geminiviridae) that infects common bean (Phaseolus vulgaris L.) and causes stunted growth and mosaic symptoms. A Toll-interleukin-1 receptor (TIR)-nucleotide-binding site (NBS)-leucine rich repeat (LRR) resistance (R) gene analog (RT4-4 gene) was cloned, using reverse transcription-PCR with degenerate NBS primers, from common bean (Phaseolus vulgaris cv. Othello) tissues undergoing a resistance response to BDMV. Northern blot and RT-PCR analyses revealed that RT4-4 was expressed and upregulated in BDMV-infected tissues after inoculation of BDMV. A functional analysis of RT4-4 was performed by generating transgenic Nicotiana benthamiana plants (susceptible to BDMV and many other viruses) and inoculating with DNA and RNA viruses. The RT4-4 transgenic plants were not resistant to BDMV. However, these plants developed a systemic necrosis phenotype in response to infection by Cucumber mosaic virus (CMV; Family Bromoviridae, Genus Cucumovirus), a tripartite positive-sense RNA virus. These plants developed a systemic necrosis response to all the CMV isolates tested, except for a bean-infecting strain (strain 67). Of all the CMV strains instead, only strain 67 systemically induced cv. Othello, causing mosaic and dwarfing symptoms. Thus, the development of the systemic necrosis response of RT4-4 transgenic N. benthamiana plants to infection with CMV strains was consistent with the relative susceptibility of cv. Othello from which the RT4-4 gene was cloned. The finding that the CMV defense response elicited by RT4-4 in N. benthamiana was very different from that in common bean, i.e., systemic necrosis versus no obvious symptoms, likely reflects the interaction of the RT4-4 protein with different host factors and/or signal transduction pathways in these two plant species. Segregation analysis of T₁ transgenic plants indicated that the CMV systemic necrosis phenotype conferred by RT4-4 acted as a single dominant gene. Agrobacterium-mediated transient expression experiments revealed that the CMV 2a protein (replicase) of the non-bean-infecting CMV strains was the elicitor of necrosis in the RT4-4 transgenic N. benthamiana plants, whereas the 2a of the bean-infecting strain (67) did not induce necrosis. A single amino acid change in the 2a of a non-bean-infecting strain, changing phenylalanine to tyrosine at position 631, abolished the necrosis phenotype. These results are consistent with RT4-4 acting in a gene-for-gene manner. Thus, RT4-4 is a CMV R gene from common bean, which is functional across plant families.